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The effect of high hydrostatic pressure on the anthocyanins of raspberry (*Rubus idaeus*)

Winai Suthanthangjai [a], Paul Kajda [a], Ioannis Zabetakis [b,*]

[a] *Procter Department of Food Science, University of Leeds, Leeds, LS2 9JT, UK*
[b] *Laboratory of Food Chemistry, Department of Chemistry, University of Athens, GR-157 71 Athens, Greece*

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Abstract

The colour stability of fruit puree made from raspberries (*Rubus idaeus*), which were subjected to high hydrostatic pressure, was studied by measuring the anthocyanin content. High hydrostatic pressure is an alternative method of food preservation to heat treatment. In this study, we assess the impact of high pressure on the colour molecules in raspberries. Fruit samples were pressured under 200, 400, 600 and 800 MPa for 15 min at a temperature controlled between 18 and 22 °C. After application of pressure, the high pressure treated samples were kept at refrigerator temperature (4 °C), room temperature (20 °C) and at 30 °C. The anthocyanin content of the raspberries was analysed after 1, 2, 4, 7 and 9 days of storage by HPLC-UV using an isocratic elution system. Two pigments were identified and quantified: cyanidin-3-glucoside and cyanidin-3-sophoroside. The highest stability of the anthocyanins was found when raspberries were pressured under 200 and 800 MPa and stored at 4 °C.

Keywords: Raspberry; High hydrostatic pressure; Anthocyanins; Colour

1. Introduction

Raspberries, known as bramble fruits, belong to the Roseceae family, genus *Rubus.* They are also called cane berries because they grow on 3–6 feet stalks with many short thorns. A raspberry is an aggregate fruit composed of bright red droplets, which are fleshy and contain seeds. Raspberries grow best in climates with cool summers and moderate winters (Deuel, 1996).

The colour of raspberries is due to the presence of anthocyanins, which are one of the most broadly distributed pigment groups in the plant world and the largest group of water-soluble pigments. In red raspberry (*Rubus idaeus*), the two anthocyanins present are derivatives of cyanidin (Fig. 1) (von Elbe & Schwartz, 1996).

The seven largest producers of raspberries in the world are the United States, Poland, the former Yugoslav Republics, Hungary, Chile, United Kingdom and France. The United Kingdom production, mostly from Scotland, is used mostly domestically in yoghurt preparations and jam products. Polish and Hungarian products are exported to Austria and Germany for juice concentrates and preservation as jams, preserves and sauces. French production is increasing dramatically and fruits are consumed fresh and also processed into preserves and fillings for the EEC market (Deuel, 1996).

From the quantitative point of view, the red raspberry fruits (*R. idaeus*) contain only 23–59 mg/l00 g of anthocyanins while black raspberries (*Rubus occidentalis*) have a very high anthocyanin content (214–428 mg/l00 g). The total anthocyanin content is, therefore, a main criterion used to differentiate between the two species. However, the number of anthocyanins present in fruits is also a criterion to distinguish between fruits. For example, pelargonidin is not present in black raspberries, is present as a trace in red raspberries (Macheix, Fleuriet, & Billot, 1990). In addition, it is also very rare to find only one glycoside. In the case of red raspberries, Cyanidin (Cy) 3-glucoside and 3-sophorosides are the major pigments

* Corresponding author. Tel.: +30-210-7274-663; fax: +30-210-7274-476.
E-mail address: izabet@chem.uoa.gr (I. Zabetakis).

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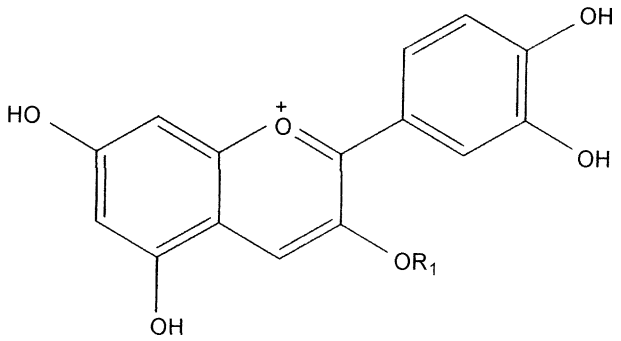

Fig. 1. The structures of cyanidin-3-glucose (R_1 = glucose) and cyanidin-3-sophoroside (R_1 = sophorose).

(Macheix et al., 1990) and Cy and pelargonidin (Pg) 3-glucosides, 3-diglucoside, 3-rutinocides, glucorutinosides, 3-sambubiosides, 3,5-diglucosides and 3,5-diglucosides and 3-rutinocide-5-glucosides are minor pigments (Jackman & Smith, 1996).

The shelf life of fresh raspberries is dependent upon time and temperature relationships and the degree of infection with fungi such as *Botrytis cinerea* and *Rhizopus*. Storage of fruits at ambient conditions will certainly reduce usable fruit, increase Howard mould count, darken the berry colour, and increase the soluble solids due to desiccation. Thus, the berries must be transported from the field to the plant as quickly as possible and they should be processed within a few hours. Factors such as weather conditions, field condition, humidity, and packing container can affect shelf life. Cooling the berries quickly and storing them at chilled storage temperatures of 2 °C and at high moisture content will delay the spoilage (Deuel, 1996).

The impact of high pressure, which is an alternative preservation method to heat treatment (Gould, 1995), on the anthocyanins of red raspberries is assessed in this work. Despite the fact that high pressure jam has been commercially available in Japan for some years (Watanabe, Arai, Kumeno, & Honma, 1991), the effect of high pressure on anthocyanins has not been studied excessively. This lack of information on the effect of high pressure on colour molecules in red raspberries prompted us to investigate the changes of the levels of anthocyanins in red raspberries after the application of different levels of high pressure.

2. Materials and methods

2.1. Solvents and anthocyanin extraction

All solvents used were of HPLC grade and purchased from Sigma (Gillingham, UK) The anthocyanin extraction was carried out as described previously (Zabetakis, Leclerc, & Kajda, 2000). Pure cyanidin-3-glucoside (Cy-3-glu) and cyanidin-3-sophoroside (Cy-3-sop) were purchased from Extrasynthese (Lyon, France) Standard solutions of these anthocyanins were prepared in the concentration range of 0.002–0.010 mg/l and they were analysed by HPLC so two standard curves were constructed and used in the analytical quantifications. All analyses were carried out in triplicate and the results are expressed as mean values.

2.2. HPLC analysis

Acetonitrile (83 ml), methanol (33 ml) and acetic acid (170 ml) were mixed with trichloroacetic acid (0.65 g) that was previously dispersed in water. The volume of this mixture was completed to 1 l with distilled water. This HPLC solvent was then degassed and used at an isocratic flow rate of 1 ml/min.

HPLC analysis was carried out using a Pye Unicam liquid chromatograph with a 4015 pump and a variable wavelength LC3 UV detector. Chromatograms were recorded on a Hewlett Packard 3394 integrator. A Partisil 5 octadecylsilane (ODS) (250×3.9 mm with particle size 5 μm, Phenomenex, Macclesfield, UK) column was coupled to a Partisil 5 guard column (30×4.6 mm with particle size 5 μm, Phenomenex, Macclesfield, UK). The sample loop was 20 μl. Detection was carried out at 520 nm.

2.3. High pressure treatment

Raspberries were subjected to four different high pressure treatments: 200, 400, 600 and 800 MPa for 15 min at a temperature of 18–22 °C. The ranges of pressure and temperature treatments used commercially are 400–600 MPa at 18–22 °C. The pressure treatments in this study have, therefore, been to chosen to include and extend the commercially used range of pressures. After this treatment, samples were stored at 4 °C, room temperature (20 °C) or 30 °C. Anthocyanin content was analysed on both untreated (blank) and high pressure treated raspberry samples. The two anthocyanins present in raspberries were identified as cyanidin-3-glucoside and cyanidin-3-sophoroside based on comparison of HPLC retention times and spectral characteristics with the corresponding standard compounds that have been purchased. Analysis was carried out immediately after the high pressure treatment and after 1, 2, 4, 7 and 9 days of storage. Each time, the levels of both Cy-3-glu and Cy-3-sop were analysed in triplicate.

3. Results

3.1. Reproducibility of anthocyanin estimation

The estimation of anthocyanins content in raspberries can be varied from extraction to extraction and from

Table 1
Concentration of Cy-3-glu and Cy-3-sop from nine different extractions and nine different injections

Extraction no.	Concentration (mg/100 g raspberry)		Injection no.	Concentration (mg/100 g raspberry)	
	Cy-3-glu	Cy-3-sop		Cy-3-glu	Cy-3-sop
1	33.4	7.38	1	31.6	6.39
2	33.7	7.56	2	31.3	6.23
3	33.1	7.56	3	31.3	6.23
4	33.4	7.38	4	31.3	6.23
5	33.4	7.38	5	31.6	6.23
6	33.9	7.19	6	31.1	6.39

batch to batch due to numerous sources of variation and errors. Thus, the reliability of the extraction and injection technique was assessed by extracting the same batch of fresh raspberries for nine times and the same extraction was injected for nine times. It can be seen in Table 1 below that there was a low level of variation from the nine extractions with both Cy-3-glu and Cy-3-sop. In addition, the nine injections from the same extraction gave the % RDS of below 2% on both peaks. This was considered to be acceptable reproducibility for this kind of analysis.

3.2. Effects of HPT on the raspberry anthocyanins after being stored at refrigerator temperature (4 °C)

At the storage temperature of 4 °C, the loss of Cy-3-glu was found to be relatively low in all samples and there is no significant difference (i.e., less than 20%) between untreated and treated samples (Table 2). However, the destruction of Cy-3-glu exhibited by 400 and 600 MPa treated samples was the greatest (25%) after 4 days of storage while the loss was minimal with raspberries treated at 200 MPa (5%). Although there is no obvious evidence of which treatment improved the stability of the Cy-3-glu on storage at refrigerator temperature, the lowest losses for up to nine days of storage of up to 18% were observed when either no pressure or 200 MPa were applied.

Similarly to Cy-3-glu, the greatest losses of Cy-3-sop on storage were found when the sample was treated at 400 MPa, with the final losses of 60% compared to 42% loss for the 200 MPa treatment and around 20% for all other treatments (Table 3). Interestingly and similarly to the case of Cy-3-glu, the lowest losses of Cy-3-sop after 9 days of storage were observed for the untreated samples.

3.3. Effects of HPT on the raspberry anthocyanins after being stored at room temperature (20 °C)

At room temperature storage, the loss of Cy-3-glu was rather similar in untreated and all HP treated

Table 2
The percentage of losses of cyanidin-3-glucoside with storage time at refrigerator temperature, at each high pressure condition investigated

Storage time (days)	Pressure applied				
	Untreated (0 MPa)	200 MPa	400 MPa	600 MPa	800 MPa
0	0	0	0	0	0
1	0.54 ± 0.04	1.38 ± 0.09	1.77 ± 0.10	0.42 ± 0.03	4.24 ± 0.25
2	4.49 ± 0.33	7.41 ± 0.55	12.6 ± 1.14	18.7 ± 1.75	14.1 ± 1.24
4	15.3 ± 1.44	5.89 ± 0.46	26.8 ± 2.10	23.3 ± 2.22	16.3 ± 1.46
7	16.8 ± 1.61	16.7 ± 1.58	38.2 ± 2.89	34.2 ± 2.11	16.4 ± 0.85
9	18.9 ± 1.11	18.6 ± 1.42	37.9 ± 2.25	33.2 ± 2.05	25.0 ± 1.19

Table 3
The percentage of losses of cyanidin-3-sophoroside with storage time at refrigerator temperature, at each high pressure condition investigated

Storage time (days)	Pressure applied				
	Untreated (0 MPa)	200 Mpa	400 MPa	600 MPa	800 MPa
0	0	0	0	0	0
1	0.11 ± 0.01	7.7 ± 0.24	0.07 ± 0.01	0.50 ± 0.03	12.2 ± 0.85
2	2.01 ± 0.14	8.11 ± 0.66	13.6 ± 0.85	16.6 ± 0.96	13.7 ± 0.55
4	4.51 ± 0.20	12.5 ± 0.77	44.2 ± 2.01	22.2 ± 1.11	17.7 ± 0.88
7	17.8 ± 0.91	15.3 ± 0.75	59.8 ± 3.01	28.0 ± 1.41	21.6 ± 1.10
9	19.2 ± 0.98	42.4 ± 2.10	62.6 ± 2.85	28.4 ± 1.44	26.8 ± 1.35

Table 4
The percentage of losses of cyanidin-3-glucoside with storage time at room temperature, at each high pressure condition investigated

Storage time (days)	Pressure applied				
	Untreated (0 MPa)	200 MPa	400 MPa	600 MPa	800 MPa
0	0	0	0	0	0
1	9.29 ± 0.55	1.38 ± 0.07	10.8 ± 0.25	3.52 ± 0.11	4.63 ± 0.16
2	12.3 ± 0.61	9.44 ± 0.71	15.6 ± 0.99	27.0 ± 2.21	14.3 ± 0.69
4	25.6 ± 2.15	10.7 ± 0.75	34.5 ± 2.56	34.1 ± 2.61	25.7 ± 2.11
7	37.7 ± 2.65	38.0 ± 2.78	50.2 ± 4.01	48.5 ± 3.74	28.3 ± 2.22
9	40.1 ± 3.01	35.0 ± 2.41	49.4 ± 3.88	46.8 ± 3.44	54.7 ± 4.07

Table 5
The percentage of losses of cyanidin-3-sophoroside with storage time at room temperature, at each high pressure condition investigated

Storage time (days)	Pressure applied				
	Untreated (0 MPa)	200 MPa	400 MPa	600 MPa	800 MPa
0	0	0	0	0	0
1	0.03 ± 0.00	10.5 ± 0.65	3.60 ± 0.17	3.53 ± 0.18	13.8 ± 0.75
2	12.4 ± 0.75	14.6 ± 0.78	19.6 ± 1.15	23.7 ± 1.24	21.4 ± 1.17
4	39.4 ± 2.01	46.5 ± 2.24	51.3 ± 2.45	41.8 ± 2.02	27.0 ± 1.39
7	64.2 ± 3.02	54.7 ± 2.54	71.2 ± 2.98	59.8 ± 2.75	48.7 ± 2.21
9	73.5 ± 3.04	65.2 ± 2.72	72.8 ± 3.04	71.3 ± 2.99	63.5 ± 2.56

samples (Table 4). However, the loss on the first day of storage was very low at HPT of 200, 600 and 800 MPa compared to untreated and HPT of 400 MPa. On the second day of storage, the loss was found to be greatest after 600 MPa treatment while 200 MPa was the lowest. From the second day of storage, it was quite obvious that, after the HPT of 600 and 400 Mpa, the degradation rates were about 10% higher than the others. Although after the HPT of 200 MPa the loss was lowest after the fourth day of storage (only 10%), the degradation rate climbed rapidly after day 7 (30%), which was the same as those of untreated samples.

In the first day of storage the untreated, 400 and 600 MPa treated samples exhibit only slight losses of Cy-3-sop (0–3%), compared to those of 200 and 800 MPa treated raspberries (10% and 15%) (Table 5). However, after the second day of storage, the loss of all samples was quite similar, being approximately 20%. After 4 days the loss of 800 Mpa treated samples was found to be around 10% lower than other samples. The total losses of all samples after nine days of storage were quite extensive, being approximately 65% on average.

3.4. Effects of HPT on the raspberry anthocyanins after being stored at 30 °C

The loss of Cy-3-glu when stored at 30 °C was quite similar in all treated samples after nine days of storage (60–65%) compared to untreated sample (80%) (Table 6). However, the initial rate of degradation of untreated and 600 MPa treated samples were found to be the highest after 2 days of storage. These two samples continued to have the greatest loss after being stored for 4 and 9 days. In contrast, the 800 MPa treated sample exhibited the smallest loss (10% lower than the rest) after one week of storage.

The initial rate of loss of Cy-3-sop was found to be nearly identical in all samples after being stored at 30 °C (Table 7). However, after 4 and 7 days of storage, the losses were found to be higher in 200 and 400 MPa treated samples, with the 800 MPa treated sample being the lowest. The total losses after nine days were found to be very high, the highest was 96% exhibited by the 400 MPa sample while the lowest was 70% from the 800 MPa sample.

Table 6
The percentage of losses of cyanidin-3-glucoside with storage time at 30 °C, at each high pressure condition investigated

Storage time (days)	Pressure applied				
	Untreated (0 MPa)	200 MPa	400 MPa	600 MPa	800 MPa
0	0	0	0	0	0
1	24.2 ± 1.25	11.9 ± 0.65	13.2 ± 0.75	4.86 ± 0.27	13.9 ± 0.77
2	29.7 ± 1.29	20.0 ± 1.11	21.1 ± 1.17	27.5 ± 1.24	18.5 ± 1.12
4	49.5 ± 1.99	37.1 ± 1.55	42.4 ± 1.62	47.3 ± 1.87	43.2 ± 1.64
7	58.9 ± 2.24	61.8 ± 2.35	62.51 ± 2.34	63.7 ± 2.38	49.9 ± 2.00
9	81.0 ± 30.4	62.8 ± 2.41	62.2 ± 2.39	65.4 ± 2.41	70.9 ± 2.74

Table 7
The percentage of losses of cyanidin-3-sophoroside with storage time at 30 °C, at each high pressure condition investigated

Storage time (days)	Pressure applied				
	Untreated (0 MPa)	200 MPa	400 MPa	600 MPa	800 MPa
0	0	0	0	0	0
1	14.0 ± 0.64	14.7 ± 0.65	13.4 ± 0.63	9.57 ± 0.57	17.7 ± 0.69
2	36.2 ± 1.80	22.3 ± 1.18	25.8 ± 1.28	24.8 ± 1.26	28.9 ± 1.32
4	45.8 ± 2.09	61.1 ± 3.08	59.4 ± 3.01	53.1 ± 2.74	39.0 ± 1.87
7	70.1 ± 3.41	75.8 ± 3.45	78.5 ± 3.56	66.5 ± 3.21	63.6 ± 3.04
9	76.6 ± 3.54	82.4 ± 3.85	90.1 ± 4.01	84.8 ± 3.94	72.7 ± 3.41

4. Discussion

High-pressure treatment has been known to have an impact on enzyme activity. Seemingly, the effect of the HPT on the anthocyanin content in raspberries is due to the changes of enzyme activity that influences the anthocyanin stability. The three enzymes known to be responsible for the anthocyanin degradation are β-glucosidase, peroxidase (POD) and polyphenoloxidase (PPO). These enzymes are believed to be affected by temperature, but a 30 °C maximum storage temperature used in this study should not have a great impact on their activities. This is simply because the optimum temperature for activity of these enzymes is known to be higher than 30 °C (Robinson & Eskin, 1991; Orruño, Owusu Apenten, & Zabetakis, 2001). However, storage at this temperature (30 °C), will surely contribute more to the enzyme-mediated losses of anthocyanins than at lower temperature storage because it is closest to their optimum temperature. In fact, the results clearly showed that the losses of anthocyanins were greater at higher storage temperatures (Tables 6 and 7).

In the present study, we found that at the storage temperature of 4 °C, the smallest percentage losses of anthocyanins were achieved, presumably due to the lowest enzyme activity at this temperature. The losses of Cy-3-glu and Cy-3-sop at refrigerator temperature after HPT of 400 and 600 MPa were higher than the others, with at 400 MPa being the highest (i.e., 35% for Cy-3-glu and 60% for Cy-3-sop after 9 days of storage) (Tables 2 and 3). This may be due to the higher remaining activity of β-glucosidase and POD after a HPT at this pressure range (Garcia-Palazon, Suthanthangjai, Kajda, Zabetakis, & peroxidase & polyphenoloxidase in red raspberry,).

When fruits were stored at room and 30 °C temperatures, that are closer to the optimum temperatures for enzymatic activity, the increased enzymatic activities lead to higher degradation of the anthocyanins. The higher losses of both pigments were observed after the HPT of 400 and 600 MPa, that are the HPTs that caused the least inactivation of the enzymes β-glucosidase, POD and PPO (Garcia-Palazon et al., XXXX).

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